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A Situation Report

NEW INTERPRETATION OF TEST FOR BRUCELLOSIS IN VACCINATED CATTLE

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NEW INTERPRETATION OF TEST FOR BRUCELLOSIS IN VACCINATED CATTLE

RESEARCH has scored again in the battle against brucellosis in cattle, by bringing a new interpretation to the long-established blood-agglutination test for diagnosing this disease. It is now possible to determine more accurately the brucellosis status of calf-vaccinated cattle without increasing the risk of spreading the disease. Results of this research have been put to work in the brucellosis eradication program.

Heretofore, many owners have been confronted with the verdict that their vaccinated animals would have to be classified as suspects or reactors when in fact these were among the most valuable animals in the herd because of their resistance to brucellosis. Blood tests of animals vaccinated as calves often presented an obstacle to complying with milk ordinances and regulations on interstate shipment. Many cattle officially vaccinated as calves that have been held for further inspection and retesting will now be given a clean bill of health.

Strain 19 Brucella abortus vaccine, made from low-virulent cultures of the organism, has been used effectively in calves to prevent brucellosis infection since it was introduced in the national brucellosis program by the U. S. Department of Agriculture in 1940. Because of the fact that an agglutinin reaction, usually temporary, is induced in most animals vaccinated as calves, which is similar to that found in infected animals, the general acceptance of calf vaccination as a disease prevention measure has been marred by the confused results after an animal reaches the age of 30 months.

Research that has been carried on for more than 2 years has established that if, in reading the results of blood tests, a higher dilution is allowed for vaccinated animals -- 1 part of blood serum to 200 parts of antigen (a suspension of Brucella organisms) rather than 1 part of serum to 100 parts of antigen, as allowed for unvaccinated animals -- the results in finding infection are comparable. That is, vaccinated animals in a great majority of cases are safe in so far as spreading brucellosis is concerned, even though they may have an agglutination titer that would require condemnation of unvaccinated animals with the same titer. There is no change in the interpretation of the test for unvaccinated animals. Results of this research were considered so immediately useful that the paper 1/ describing them was duplicated and sent to State and Federal disease control officials in advance of its publication in technical journals.

1/ "Relationship of Sero-Agglutinin Titers to Udder Infection in Strain 19 Vaccinated Cattle," by Edwin R. Goode, T. E. Amerault, and C. A. Manthei.

The research was done by scientists of the Agricultural Research Service at Beltsville, Md., in cooperation with disease control officials in Washington, D.C., Illinois, and New York.

THE RESEARCH

RECOVERY of Brucella organisms from the milk of infected cows is an established practice among scientists in demonstrating positive proof of brucellosis. It is especially effective in chronic cases of the disease which are with few exceptions characterized by infection of the udder. The plan of research therefore called for the isolation of Brucella bacteria from udder samples, then correlating the results of this search with the testing of blood from the same animals.

Most of the previous research with udder samples had been made, however, with those from unvaccinated animals. This series of tests put more emphasis on vaccinated animals. To make the research more complete, separate samples were collected from each of the four quarters of the udder. Composite sampling from all four quarters might have resulted in the organisms becoming so diluted that they would not be recovered.

To begin with, the work was done in an area where calf vaccination has been a regular practice for many years. Jersey County, Ill., part of the St. Louis milkshed, was chosen; calves have been vaccinated there since 1942. Later, to make the results more comprehensive, a decision was reached to investigate infection in animals that had had every kind of brucellosis treatment, ranging from no control at all to a strict test-and-eradication plan. New York State offered all of these conditions. Altogether, milk and blood samples were taken from 637 vaccinated and 103 unvaccinated cattle in 278 herds containing 6,276 adult cows. Of these, 263 were in Jersey County, Ill., and 477 were at scattered locations in New York.

Each quarter sample of milk was injected into guinea pigs, which were kept isolated for 35 days, then sacrificed, autopsied, blood tested and cultured for evidence of brucellosis infection. Other portions of the same samples were placed on a suitable medium in Petri dishes and incubated to see if Brucella colonies developed. Many tests were applied to these colonies, for identification and classification of the organisms.

Clots were removed from the blood samples and the remaining blood serum was subjected to the standard tube and plate agglutination tests. In the tube method, as ordinarily carried out in most States, 2 cubic centimeters of antigen is placed in each of three test tubes. Serum is added in the amounts of .04, .02, and .01 cubic centimeters, making dilutions of 1 to 50, 1 to 100, and 1 to 200. (In this more exhaustive series of tests, ten or more different dilutions were used.) The tubes are incubated for 48 hours and readings are made. The titer of the sample -- titer is defined as the greatest dilution at which agglutination takes place -- is determined from this reading. Agglutination is a visible clumping of the antibody in

the serum with the antigen, which is made of Brucella organisms. Results showed that the alternate interpretation of agglutination reactions may be applied to either the tube or the quicker plate test with equal efficiency.

These tests, after matching them with milk-sample findings from the same animals, showed that

- Infection in unvaccinated animals, as in the past, is indicated by a titer of 1:100 or higher, and

- Infection in vaccinated animals is indicated by a titer of 1:200 or higher.

Closely associated with the second finding is the fact that all but 1 of 51 positively infected cows were from herds that had one or more animals with a maximum titer of 1:400 or higher -- a "hot" infection.

The new interpretation, by more accurately defining the infection status of vaccinated animals,

- Reduced by three times the number of reactors,

- Reduced by nearly one-half the number of suspects, and

- Increased by five times the number of animals testing negative.

In addition to these main lines of research, several contributory lines of investigation developed, the results of which have value in the brucellosis eradication campaign:

- The milk ring test, which was previously developed as a practical and economical method of herd testing, is confirmed as "an effective diagnostic aid in the control of bovine brucellosis." In every case but one where Brucella bacteria were found, the milk was positive to the ring test, in herd samples. This test also classified as infected the most dangerous herds -- those having animals with a titer of 1:400 or higher. Furthermore, calfhood vaccination when made at the recommended age does not materially affect the results of the ring test.

- Calfhood vaccination is confirmed as a valuable method of brucellosis control. In Jersey County, Ill., when vaccination started in 1942, the percentage of reactors tested was 5 percent. Five years later, in 1947, it had dropped to 3.7 percent. By 1952 it was down to 0.5 percent. In these tests, 29 of 637 vaccinated cattle, or only 4.5 percent, tested positive, whereas 22 of 103 unvaccinated cattle, or 21.3 percent, tested positive.

- The recommended age of calfhood vaccination -- 6 to 8 months -- is reemphasized. The titers of 88 heifers vaccinated at 8 months and those of 73 heifers vaccinated at 12 to 15 months were taken when all the animals reached the age of 30 months. At that

time, the titers of 95.5 percent of the heifers vaccinated at 8 months had dropped below 1:100, where they would not interfere with the new alternate interpretation of blood tests for vaccinated animals. The remaining animals would be rated as suspects. Of those vaccinated as yearlings, only 43.8 percent had dropped below a titer of 1:100, hence more than half of them would be rated as suspects or reactors according to this interpretation.

¶ Herd replacements must be purchased with great care, to insure that they are brucellosis-free. In these tests, it was found that 73 percent of the 26 herds having infected cattle had been supplemented by purchased replacements. This indicates that the great majority of infection had been bought and paid for.

PUTTING THE RESEARCH TO WORK

THESE RESEARCH RESULTS were presented at the annual meeting of the U.S. Livestock Sanitary Association at Omaha in November 1954. The Brucellosis Committee recommended, and the Association unanimously adopted, a recommendation that the new findings be written into the Association's Uniform Methods and Rules for the Establishment and Maintenance of Certified Brucellosis-Free Herds of Cattle and Modified Certified Areas. The committee praised the work of the research men:

"We commend those who have done the ... research.... Each year, as our research people bring us new information, we are able to make adjustments which bring us nearer to our goal ... eliminating for slaughter the last infected animal."

The Animal Disease Eradication Branch, Agricultural Research Service, submitted the recommendations to officials in cooperating States and thereafter issued them as instructions to the field.

Two tables, somewhat modified from those sent to the field, are given for interpretation of reactions of the blood-serum agglutination test.

Table 1.-- For unvaccinated cattle (unchanged)

Dilutions			Diagnosis
1:50	1:100	1:200	
-*	-	-	Negative
I	-	-	Suspect
+	-	-	Suspect
+	I	-	Suspect
+	+**	-	Reactor

Table 2.-- For calf-vaccinated cattle (new interpretation)

Dilutions			Diagnosis
1:50	1:100	1:200	
+*	-	-	Negative
+	I	-	Suspect
+	+	-	Suspect
+	+	I	Suspect
+	+	+**	Reactor

Key to both tables:

- = Negative
+ = Complete agglutination
I = Incomplete agglutination
* = or less
** = or higher

THE PROSPECT

IN PRACTICE, a number of advantages and changes of emphasis are expected to accrue from field use of the new interpretation:

- Farmers will learn more quickly whether or not they have brucellosis-free herds.
- Dairy herds will qualify more readily under present and proposed milk ordinances.
- Thousands of vaccinated animals will be saved from condemnation as brucellosis reactors.
- Less of the manpower engaged in brucellosis eradication will be required to keep fewer suspected animals under observation.
- Increased interest will be shown in calfhood vaccination.
- The recommended age of calfhood vaccination -- 6 to 8 months -- will be stressed.
- Proper branding or tattooing of vaccinated animals will take on new importance, for each animal must carry evidence of vaccination.
- Some shipping problems across State lines will be reduced.